Journal of Forensic and Legal Medicine 20 (2013) 174–178

Contents lists available at SciVerse ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Case report

The identification of elephant ivory evidences of illegal trade with mitochondrial cytochrome b gene and hypervariable D-loop region

Eun-jung Lee Ph.D [a], Yang-han Lee Ph.D [a], Seo-hyun Moon M.Sc [a], Nam-ye Kim Dr. [a], Soon-hee Kim Ph.D [b], Moon-sik Yang Ph.D [c], Dong-ho Choi Ph.D [a], Myun-soo Han Ph.D [a,*]

[a] Forensic DNA Center, National Forensic Service, 331-1 Shinwol 7-dong, Yangcheon-gu, Seoul 158-707, Republic of Korea
[b] Eastern District Office, National Forensic Service, Wonju 220-805, Gangwon Province, Republic of Korea
[c] Biological Sciences, Chonbuk National University, Chonju 561-756, Jeolla province, Republic of Korea

ARTICLE INFO

Article history:
Received 31 October 2011
Received in revised form
11 June 2012
Accepted 17 June 2012
Available online 27 July 2012

Keywords:
Ivory
Illegal trade
CITES
Elephant
Cytochrome b
D-loop

ABSTRACT

DNA analysis of elephant ivory of illegal trade was handled in this work. The speciation and geographical origin of nine specimens of elephant ivory were requested by the police. Without national authorization, the suspect had purchased processed ivory seals from January to May, 2011 by Internet transactions from a site in a neighboring country. The DNA of decalcified ivory evidences was isolated with QIAGEN Micro Kit. The total 844–904 base pair sized sequences of mitochondrial cytochrome b and D-loop region could be acquired using direct sequencing analysis. They were compared with the sequences registered in GenBank. It was confirmed that most specimens were likely from African forest elephants (*Loxodonta cyclotis*), one from African savanna elephant (*Loxodonta africana*) and one from Asian elephant (*Elephas maximus*). Analysis of the mitochondrial hypervariable D-loop region sequence of elephants verified that one African savanna elephant might be from South Africa and one Asian elephant from Laos. Cytochrome b and D-loop region located in the mitochondrial DNA resulted in the successful determination of elephant DNA from nine processed ivory specimens.

© 2012 Elsevier Ltd and Faculty of Forensic and Legal Medicine. All rights reserved.

1. Introduction

The ivory trade is the main reason for the decline in numbers of elephants such as African elephants (*Loxodonta cyclotis* and *Loxodonta africana*) and Asian elephant (*Elephas maximus*). The species identification of the ivory evidences is required for the enforcement of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) regulations. Beginning in 1993 year, CITES regulations have come into effect in South Korea. Some Asian communities carve their personal seal on the end of ivory cylinders to be used as a prestigious stamp.[1,2] These ivory products are derived from the core of the tusk and require a decalcification step before their DNA extraction.

Several conserved primers developed in mitochondrial DNA (mtDNA) regions such as cytochrome b,[3] 12S ribosomal RNA (rRNA),[4] 16S rRNA[5] and the hypervariable D-loop region[3,6] have been used for species identification and phylogenetic studies. This genetic marker analysis provides sensitive and useful tools for prevention of illegal hunting, poaching, and trade. The short-sized fragments of partial cytochrome b aid in species identification of highly processed or degraded forensic evidence.[7] The hypervariable D-loop region of mtDNA is particularly suitable for the genetic analysis of populations and closely related taxa.[8,9]

In the present case, police arrested an Internet-based ivory distributor, who had sold ivory products from January to May, 2011. The felon had purchased the ivory products from online shop in a neighboring country without any national permission. The seized ivory specimens against CITES regulations were tested to identify their species and geographical origins using partial cytochrome b and D-loop region.

2. Materials and methods

2.1. Specimens

Photographs of the nine ivory items seized by police are shown at Fig. 1. The ivory discs of about 50 mm in height were cut off with a dental drill and these discs were treated with 10–20 ml of 0.5 M EDTA for ten days. Chopped ivory (330 mg–500 mg) was treated with 1 ml of ATL and 100 μl of protease K in a QIAamp DNA Micro kit (QIAGEN, Valencia, CA, USA). DNA was extracted from 500 μl of the lysate. The elution volume was 80 μl. Amount and purity of extracted DNA was quantitated using a NanoDrop 2000 UV/vis

* Corresponding author. Tel.: +82 2 2600 4840; fax: +82 2 2600 4866.
E-mail address: hmyunsoo@korea.kr (M.-s. Han).

1752-928X/$ – see front matter © 2012 Elsevier Ltd and Faculty of Forensic and Legal Medicine. All rights reserved.
http://dx.doi.org/10.1016/j.jflm.2012.06.014

Fig. 1. The ivory evidence. The item number (1–6) is inscribed under evidence. The lengths of the bottom plate of item 1 and item 2 are 3.3 cm × 1.35 cm. Items 3 and 4 have hexahedral shapes and their bottom length is 1.55 cm × 1.55 cm and 1.7 cm × 1.7 cm, respectively. Items 5 and 6 are round cylinders. The diameters of the two item 5 specimens are 1.1 cm and those of item 6 are 1.5 cm and 1.2 cm. The height of all items is about 5–7.5 cm.

spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The concentration for each evidence was as follows: item 1 (6.4 ng/μl), item 2 (4.8 ng/μl), item 3 (1.6 ng/μl), item 4-1 (6.8 ng/μl), item 4-2 (9.2 ng/μl), item 5-1 (11.3 ng/μl), item 5-2 (7.4 ng/μl), item 6-1 (3.1 ng/μl), item 6-2 (6.7 ng/μl). The average ratio of A260 to A280 was 1.24. There was not any positive control which available for an internal laboratory control for these ivory items.

2.2. Molecular markers and PCR amplification

The partial cytochrome b gene from 14850 to 15149 (numbered according to the human mtDNA sequence) was amplified by cyb-F and cytb-R (Table 1).[3,7] A segment of mtDNA including C terminal region of cytochrome b, the next threonine and proline tRNAs, and the rest noncoding control region of D-loop was amplified by MDL3 and MDL5[8] in order to discover the geographical origin of items. In case of samples could not be amplified with MDL3 and MDL5, primers AFDL1 ~ AFDL4 were used for their amplification.[9] Both PCR amplifications were performed in a 50 μl reaction mixture, which contained 16–92 ng of extracted DNA, 20 μM of each of primers, 2.5 unit of AmpliTaq DNA polymerase and reaction buffer of Gold ST★R 10X buffer (PROMEGA, Madison, WI, USA). Amplification was conducted in a 9700 thermal cycler (Applied Bio-Systems, Foster city, CA, USA) with 40 cycles of each amplification reaction.[3,7–9] All amplification reactants included negative controls that no DNA was added. PCR products were checked on a 2.5% agarose gel and purified with a QIAquick PCR purification Kit (QIAGEN).

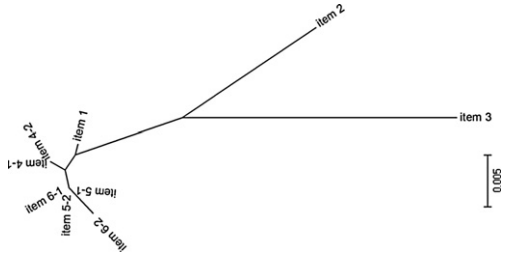

Fig. 2. The radial phylogenetic tree based on cytochrome b gene of nine ivory evidences by neighbor-joining method.

2.3. Sequencing analysis and BLAST search

The purified PCR products were sequenced directly using the aforementioned primers with an ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied BioSystems). The unincorporated

Table 1
Primer sequences used in this case study.

Gene	Primer name	Primer sequence	Fragment length	Ta	Ref
Cytb	Cytb-F	5′-CCA TCC AAC ATC TCA GCA TGA TGA Aa-3′	357 bp	50 °C	3,7
	Cytb-R	5′-CCC TCA GAA TGA TAT TTG TCC TCA-3′			
D-loop	MDL3	5′-CCC ACA ATT AAT GGG CCC GGA GCG-3′	630 bp	63 °C	8
	MDL5	5′-TTA CAT GAA TTG GCA GCC AAC CAG-3′			
	AFDL1	5′-TTA CAC CAT TAT CGG CCA AAT AG-3′	400 bp	55 °C	9
	AFDL2	5′-TGA CAC ATT GAT TAA ACA GTA CTT GC-3′			
	AFDL3	5′-CTT CTT AAA CTA TTC CCT GCA AGC-3′	377 bp	58 °C	
	AFDL4	5′-GTT GAT GGT TTC TCG GAG GTA G-3′			

Ta: annealing temperature; Ref: reference.

Table 2
The polymorphic sites for the 357 bp fragment of the cytochrome b gene from nine specimens.

Specimens	0 2 7	0 6 3	0 7 6	0 9 3	0 9 6	1 0 2	1 3 8	1 4 7	1 5 0	1 9 5	2 3 1	2 4 6	2 6 1	2 6 7	2 7 6	2 9 7	3 0 6	3 3 3
Item 1	C	G	C	T	T	C	C	T	A	T	G	A	G	C	C	C	C	G
Item 2	T	A	T	.	.	.	T	.	G	C	A	G	.	T	.	.	.	.
Item 3	–	A	T	.	C	T	.	C	C	.	A	.	A	.	A	T	.	A
Item 4-1	T	.	.	C	.	.	.	.	.	.	A	.	.	.	.	.	.	.
Item 4-2	T	.	.	C	.	.	.	.	.	.	A	.	.	.	.	.	.	.
Item 5-1	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Item 5-2	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Item 6-1	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Item 6-2	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	T	.

A period denotes a matching base with the top-most sequence. "–" means the site failed to obtain.

Table 3
Taxonomic identifications of nine items with cytochrome b gene.

Specimen	Fragment size in bp	NCBI AC #	Base similarity (homology,%)	Species	Appendix in CITES
Group 1: Item 1	357	AY359273	356 bp/357 bp (99%)	*Loxodonta cyclotis*	NE
Group 2: Item 2	357	AB362887	356 bp/357 bp (99%)	*Loxodonta africana*	I or II
Group 3: Item 3	297	AJ428946	297 bp/297 bp (100%)	*Elephas maximus*	I
Group 4: Item 4-1 and 4-2	357	AY768855	356 bp/357 bp (99%)	*Loxodonta cyclotis*	NE
Group 5: Item 5-1, 5-2, 6-1	357	AY768855	357 bp/357 bp (100%)	*Loxodonta cyclotis*	NE
Group 6: Item 6-2	357	AY768855	356 bp/357 bp (99%)	*Loxodonta cyclotis*	NE

Fragment size in bp: The 5′ region of 60 bp from item 3 could not be acquired, and so 297 bp fragment was compared with the database in NCBI; "NCBI AC #" means accession number in NCBI which shows high homology with our evidences; "bp" means base pair; In base similarity column, fragment size acquired by cytochrome b primer pairs is located at the right side of the slash and fragment size matched in NCBI is located at the left side; "NE" means not evaluated.

fluorescent dyes were eliminated using a DyeEx 2.0 Spin Kit (QIAGEN). Automated DNA sequencing was performed using an ABI 3500 sequencer (Applied Biosystems). MEGA 5.0 software was used for the sequence arrangements, the construction of Neighbor-joining tree and the calculation of evolutionary genetic distances.[10] A comparison was made between our secured sequences and the database of National Center for Biotechnology Information (NCBI) using nucleotide–nucleotide The Basic Local Alignment Search Tool (BLASTn).[11]

3. Results and discussion

3.1. Species identification with cytochrome b gene

The amplified products and nucleotide sequences of mitochondrial cytochrome b were successfully acquired by the first PCR reactions. Any secondary amplification was not needed. But we could not obtain the 5′ region of 60 bp of 357 bp sized cytochrome b from item 3. The nucleotide sequences of cytochrome b region

Table 4
The polymorphic sites for the 547 or 546 bp fragment of the cytochrome b, two tRNAs and control regions of nine specimens.

	005	014	026	032	036	038	044	051	071	094	106	128	130	190	214	216	219	220	224	235
Item 1	C	T	T	T	T	G	T	A	T	C	A	T	T	A	A	C	A	C	C	T
Item 2	A	.	C	C	C	.	.	G	.	T	G	C	.	.	G	.	–	T	.	.
Item 3	A	C	.	.	C	A	C	.	C	.	.	C	C	.	.	T	.	.	T	C
Item 4-1	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Item 4-2	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Item 5-1	.	C	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.
Item 5-2	.	C	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.
Item 6-1	.	C	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.
Item 6-2	.	C	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.

	257	271	316	376	386	396	457	488	538	893	907	001	003	415	511	613	644	704	805	975
Item 1	T	T	T	A	C	T	G	T	C	A	T	A	G	C	T	A	G	A	T	A
Item 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	G	.	.	.	G
Item 3	C	A	.	G	T	C	.	.	T	G	C	.	.	T	C	.	.	G	C	.
Item 4-1	.	.	C	.	T	C	A	C	.	.	.	G	A	.	C	.	.	.	.	.
Item 4-2	.	.	C	.	T	C	A	C	.	.	.	G	A	.	C	.	.	.	.	.
Item 5-1	.	.	C	.	T	C	A	C	.	.	.	G	A	.	C	.	.	.	.	.
Item 5-2	.	.	C	.	T	C	A	C	.	.	.	G	A	.	C	.	.	.	.	.
Item 6-1	.	.	C	.	.	C	A	C	.	.	.	.	A	.	C	.	.	.	.	.
Item 6-2	.	.	C	.	T	C	A	C	.	.	.	G	A	.	C	.	A	.	.	.

	480	488	499	502	511	533	533	603
Item 1	A	A	T	C	C	C	G	C
Item 2	.	.	C	.	.	T	.	.
Item 3	G	G	.	T	T	.	.	T
Item 4-1	.	.	C	.	.	.	A	.
Item 4-2	.	.	C	.	.	.	A	.
Item 5-1	.	G	.	.	T	.	A	.
Item 5-2	.	.	.	.	.	.	A	.
Item 6-1	.	G	.	.	T	.	A	.
Item 6-2	.	G	.	.	T	.	A	.

A period denotes a matching base with the top-most sequence. "–" means the deletion site.

Table 5
Evolutionary genetic distances calculated from a segment of mtDNA includes C terminal region of cytochrome b, the next threonine and proline tRNAs and the rest noncoding control region of D-loop of nine specimens.

	Item 1	Item 2	Item3	Item4-1	Item4-2	Item5-1	Item5-2	Item6-1	Item6-2
Item 1									
Item 2	0.028								
Item 3	0.054	0.069							
Item 4-1	0.021	0.042	0.061						
Item 4-2	0.021	0.042	0.061	0.000					
Item 5-1	0.024	0.050	0.056	0.007	0.007				
Item 5-2	0.021	0.046	0.061	0.004	0.004	0.004			
Item 6-1	0.021	0.046	0.056	0.011	0.011	0.004	0.007		
Item 6-2	0.026	0.052	0.059	0.009	0.009	0.002	0.006	0.006	

Table 6
Taxonomic identifications of nine items with a segment of mtDNA including C terminal region of cytochrome b, the next threonine and proline tRNAs and the rest noncoding control region of D-loop.

Specimen	Fragment size in bp	NCBI AC #	Base similarity (homology, %)	Species	Appendix in CITES
Group 1: Item 1	547	AF527650	546 bp/547 bp (99%)	*L. africana*	I or II
Group 2: Item 2	546	AF219244	546 bp/546 bp (100%)	*L. africana*	I or II
Group 3: Item 3	547	HQ113847	547 bp/547 bp (100%)	*E. maximus*	I
Group 4: Item 4-1 and 4-2	547	AF527640	543 bp/547 bp (99%)	*L. africana*	I or II
Group 5: Item 5-1	547	AF527640	547 bp/547 bp (100%)	*L. africana*	I or II
Group 6: Item 5-2	547	AF527640	545 bp/547 bp (99%)	*L. africana*	I or II
Group 7: Item 6-1	547	AY359271	547 bp/547 bp (100%)	*L. cyclotis*	NE
Group 8: Item 6-2	547	AF527640	546 bp/547 bp (99%)	*L. africana*	I or II

NCBI AC #: accession number in NCBI, which shows high homology with our evidences; bp: base pair; Base similarity: fragment size acquired by D-loop primer pairs is located at the right side of the slash and fragment size matched in NCBI is located at the left side; NE: not evaluated.

from all specimens were aligned and compared using MEGA 5.0 software. The compared results of 357 bp sized sequences from 8 specimens and 297 bp sized sequence from one specimen (item 3) were summarized in Table 2 and Fig. 2. Eighteen nucleotide substitutions were detected (Table 2), but any difference was not found at amino acid sequences in the translated form of vertebrate mitochondria. Six haplotypes were detected (Table 3). They were queried using BLASTn in NCBI. Cytochrome b sequences acquired from seven specimens exhibited 99.7–100% homology with those (accession number of AY359273 and AY768881 in NCBI) of *L. cyclotis* (African forest elephant). Cytochrome b from item 2 exhibited 99.7% homology with AB362887 from *L. africana* (African savanna elephant). Cytochrome b from item 3 exhibited 100% homology with AJ428946 from *E. maximus* (Asian elephant). Since 1986, *E. maximus* has been listed as the Endangered (abbreviated as EN, high risk of extinction in the wild) by The International Union for Conservation of Nature (IUCN), the world's main authority on the conservation status of species.[12] *L. africana* or African savanna elephant was designated as the Vulnerable (VU, high risk of endangerment in the wild) by ICUN. *E. maximus* and *L. africana* (except the populations of Botswana, Namibia, South Africa and Zimbabwe, which are included in Appendix II) are listed on Appendix I of the CITES.[13]

3.2. Geographical origin identification with D-loop region

A segment of mtDNA includes C terminal region of cytochrome b, the next threonine and proline tRNAs, and the rest noncoding control region of D-loop was successfully analyzed with three primer pairs. The nucleotide sequences of D-loop region from item 2, 3 and 4-2 could be acquired using MDL primer pair. Sequences of other items were acquired by AFDL primer pairs. Based on this region, 48 polymorphic sites of eight haplotypes were detected (Tables 4 and 5). The item 2 exhibited 100% homology with AF219244 (region 18–563) of South Africa sample (Table 6), AF219242 (49–594) and AJ224821 (15207–15752) of *L. africana*. Item 2 might be included in Appendix II of CITES. The item 3 exhibited 100% homology with HQ113847 (region 29–575, haplotype LaoPDR-A) of Laos country sample, AY245822 (30–576, haplotype AE) and AY245820 (30–576, haplotype AH) of *E. maximus*. Item 3 might be included in Appendix I of CITES. The item 5-1 and item 6-1 had 100% homology with *L. africana* and with *L. cyclotis*, respectively. But, between item 2 and 5-1, base substitutions was detected 26 times and between item 5-1 and 6-1, base substitutions were detected twice (Table 4). The genetic distances of item 2 and item 5-1, which showed high homology with *L. africana*, was 0.05, and that of item 5-1 and item 6-1 was 0.004 (Table 5).

4. Conclusion

We tested the identification of species and geographical origin with cytochrome b universal primer and elephant control region primers without any positive elephant control. Relatively short cytochrome b was successful in acquirement sequences from nine of the ivory items, but long control region including 3′ cytochrome b region from all items was not acquired using the primer pair MDL3 and MDL5. Shortly designed primers for control region such as AFDL1∼4, were helpful for the successful amplification. Lee et al. tested successfully the species identification of 382 out of 453 ivory samples using the partial sequence of the cytochrome b gene within the 402, 365, and 188 bp fragments.[14] Gupta group also used shortly designed primers of D-loop region for the identification of the forensic samples of elephant origin.[15,16] Except for item 2, 3 and 6-1, there were species inconstancies in other items resulted from BLAST search. Base substitutions and evolutionary genetic distances among control region sequences acquired from nine specimens showed that item 1, 4-1, 4-2, 5-1, 5-2 and 6-2 had high homologies with African forest elephant. The species identification based on mitochondrial DNA alone is

problematic for the Elephantidae because elephant females stay with their natal core social group (or herd) and females do not typically migrate between herds.[17] But elephant males disperse from the natal herd and mediate nuclear gene flow between herds. It was hypothesized that these sex differences in elephants enabled the observed phylogeographic incongruence in mito-nuclear patterns.[18,19] Mitochondrial DNA phylogeographic patterns among elephantids are often discordant with those of nuclear DNA markers and morphology.[19] In Rohland et al.'s study, comparative nuclear sequence data set concluded that African savanna and forest elephants should be classified as two species.[20] Ishida group studied short nuclear DNA sequences for distinguishing African forest and savanna elephants.[21] In forensic fields with small amount of DNA, the primary method for species identification is mtDNA analysis. If we use nuclear markers which can detect DNA from low quality or quantity template, it is possible to get more specific result for elephant ivory identification. Decalcified samples could be applied in the identification of processed products of elephant ivory.

Ethical approval

None.

Funding

This work was funded by National Research Foundation of Korea (2011-0027734).

Conflicts of interest

None.

References

1. Scigliano E. *Love, war, and circuses: the age-old relationship between elephants and humans*. Boston: Houghton Mifflin; 2002.
2. Wasser SK, Mailand C, Booth R, Mutayoba B, Kisamo E, Clark B. Using DNA to track the origin of the largest ivory seizure since the 1989 trade ban. *Proc Natl Acad Sci USA* 2007;**104**:4228–33.
3. Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc Natl Acad Sci USA* 1989;**86**:6196–200.
4. Prakash S, Patole MS, Ghumatkar SV, Nadode SD, Shinde BM, Shouche YS. Mitochondrial 12S rRNA sequence analysis in wildlife forensics. *Curr Sci* 2000;**78**:1239–41.
5. Guha S, Goyal SP, Kashyap VK. Genomic variation in the mitochondrially encoded cytochrome b (MT-CYB) and 16S rRNA (MT-RNR2) genes: characterization of eight endangered Pecoran species. *Anim Gene* 2006;**37**:262–5.
6. Eichmann C, Parson W. Molecular characterization of the canine mitochondrial DNA control region for forensic applications. *Int J Leg Med* 2007;**121**:411–6.
7. Barallon R In: Patrick J, Thomson J, editors. *Species determination by analysis of the cytochrome b gene in forensic DNA profiling protocol*. New Jersey: Humana; 1998. p. 251–60.
8. Fernando P, Pfrender ME, Encalada SE, Lande R. Mitochondrial DNA variation, phylogeography and population structure of the Asian elephant. *Heredity* 2000;**84**:362–72.
9. Eggert LS, Rasner CA, Woodruff DS. The evolution and phylogeography of the African elephant inferred from mitochondrial DNA sequence and nuclear microsatellite markers. *Proc R Soc Lond* 2002;**269**:1993–2006.
10. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximun likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evo* 2011;**28**:2731–9.
11. Altschul SF, Madden TL, Alejandro AS, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997;**25**:3389–402.
12. http://www.iucnredlist.org/apps/redlist/details/12392 or 7140 IUCN red list of threatened species; 2010.
13. http://www.cites.org/eng/app/appendices.shtml.
14. Lee JC, Hsieh HM, Huang LH, Kuo YC, Wu JH, Chin SC, et al. Ivory identification by DNA profiling of cytochrome b gene. *Int J Leg Med* 2009;**123**:117–21.
15. Gupta SK, Thangaraj K, Singh L. A simple and inexpensive molecular method for sexing and identification of the forensic samples of elephant origin. *J Forensic Sci* 2006;**51**:805–7.
16. Gupta SK, Thangaraj K, Singh L. Identification of the source of ivory idol by DNA analysis. *J Forensic Sci* 2011;**56**:1343–5.
17. Archie EA, Hollister-Smith JA, Poole JH, Lee PC, Moss CJ, Maldonado JE, et al. Behavioural inbreeding avoidance in wild African elephants. *Mol Ecol* 2007;**16**:4138–48.
18. Roca AL, Georgiadis NJ, O'Brien SJ. Cyto-nuclear genomic dissociation and the African elephant species question. *Quaternary International* 2007;**169–170**:4–16.
19. Ishida Y, Oleksyk TK, Georgiadis NJ, David VA, Zhao K, Stephens RM, et al. Reconciling apparent conflicts between mitochondrial and nuclear phylogenies in African elephants. *PLOS One* 2011;**6**:e20642.
20. Rohland N, Reich D, Mallick S, Meyer M, Green RE, Georgiadis NJ, et al. Genomic DNA sequences from mastodon and woolly mammoth reveal deep speciation of forest and savanna elephants. *PLOS Biol* 2010;**8**:e1000564.
21. Ishida Y, Demeke Y, van Coeverden de Groot PJ, Georgiadis NJ, Leggett KE, Fox VE, et al. Distinguishing forest and savanna African elephants using short nuclear DNA sequences. *J Hered* 2011;**102**:610–6.